

REMARKS

Entry of the above amendment prior to examination is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims. The attached pages are captioned "**Version with Markings to Show Changes Made.**"

I. Amendments

The specification has been amended in accordance with 37 C.F.R. §1.821 through 1.825 to add the Sequence Listing.

The specification has further been amended to remove embedded hyperlinks in accord with MPEP §608.01.

The specification has further been amended to correct a typographical error.

The specification and claims have been amended in accordance with 37 C.F.R. §1.821(d) to add SEQ ID NO:s.

No new matter is introduced by way of these amendments.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 846-7615.

Respectfully submitted,

Date:

4/25/02

Victoria L. Boyd

Victoria L. Boyd
Registration No. 43,510

Genencor International, Inc.
925 Page Mill Road
Palo Alto, CA 94304
Phone: (650) 846-7615
Facsimile (650) 845-6504

Version with Markings to Show Changes Made

In the specification:

On page 7, please replace the paragraph starting on line 5 with the following:

Figure 2 shows the predicted amino acid sequence (SEQ ID NO:2) based on the nucleotide sequence provided in Figure 1 (SEQ ID NO:2).

On page 18, please replace the paragraph starting on line

A Basic BLASTN search ([<http://www.ncbi.nlm.nih.gov/BLAST>) of the non-redundant nucleic acid sequence database was conducted on September 11, 2001, with the *bgl4* gene sequence presented in Figure 1 (SEQ ID NO:1), indicated no sequences producing significant alignments (i.e. with an E value of less than 10^{-5}).

On page 23, please replace the paragraph starting on line 31 with the following:

Figure 2 shows the predicted amino acid sequence (SEQ ID NO:2) of an exemplary BGL4 polypeptide based on the nucleotide sequence provided in Figure 1 (SEQ ID NO:1). The predicted molecular weight of the encoded BGL4 polypeptide is 90.7kDa. No sequence resembling a signal peptide (Nielsen, H., Engelbrecht, J., Brunak, S., von Heijne, G., Protein Engineering, 10:1-6, 1997) is present at the amino terminus of BGL4 suggesting that the BGL4 polypeptide is not secreted.

On page 24, please replace the paragraph starting on line 1 with the following:

A Basic BLASTP search ([<http://www.ncbi.nlm.nih.gov/BLAST>) of the non-redundant protein database, conducted on September 11, 2001 with the BGL4 amino acid sequence indicated 46% sequence identity to GenBank Accession Number X05918 (beta-glucosidase precursor of *Kluyveromyces marxianus*), 45% sequence identity to GenBank Accession Number AL355920 (beta-glucosidase precursor of *Schizosaccharomyces pombe*), 42% sequence identity to GenBank Accession Number AF329731 (beta-glucosidase of *Volvariella volvacea*), and 41% sequence identity to GenBank Accession Number AJ293760 (putative beta-glucosidase of *Agaricus bisporus*). The ten sequences having highest identity but less than 46% identity with BGL4 were all annotated as beta-glucosidases. These sequence similarities indicate that BGL4 is a member of glycosyl hydrolase family 3 (Henrissat, B. and Bairoch, A. (1993) Biochem. J. 293:781-788).

On page 28, please replace the paragraph starting on line 26 with the following:

Preferred culture conditions for a given filamentous fungus may be found in the scientific literature and/or from the source of the fungi such as the American Type Culture Collection (ATCC; "[http://]www.atcc.org/"). After fungal growth has been established, the cells are exposed to conditions effective to cause or permit the over expression of BGL4.

On page 37, please replace the paragraph starting on line 19 with the following:

Exemplary computer programs which can be used to determine identity between two sequences include, but are not limited to, the suite of BLAST programs, e.g., BLASTN, BLASTX, and TBLASTX, BLASTP and TBLASTN, publicly available on the Internet at [http://]www.ncbi.nlm.nih.gov/BLAST/. See also, Altschul, *et al.*, 1990 and Altschul, *et al.*, 1997.

On page 40, please replace the paragraph starting on line 9 with the following:

The *T. reesei* RNA is used as template for RT-PCR using methods known in the art (Loftus, J. et al., Science, 249:915-918, 1990). During this procedure the mRNA is reverse transcribed to produce first strand cDNA. The cDNA subsequently serves as template for PCR amplification of *bgl4* cDNA sequences using specific oligonucleotide primers designed in accordance with SEQ ID No. 1 or SEQ ID No. [4]3.

In the claims:

2. An isolated polynucleotide selected from the group consisting of:
 - (a) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
 - (b) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
 - (c) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 95% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
 - (d) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having the amino acid sequence presented in Figure 2 (SEQ ID NO:2);

- (e) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;
- (f) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having the amino acid sequence presented as SEQ ID NO:2;
- (g) a nucleic acid sequence presented as SEQ ID NO:3, or the complement thereof; and

a nucleic acid sequence that hybridizes, under high stringency conditions to the sequence presented as SEQ ID NO:3, or the complement or a fragment thereof, wherein said isolated polynucleotide encodes a polypeptide having the biological activity of a β -glucosidase.

8. An expression construct including a polynucleotide sequence (i) having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2), or (ii) being capable of hybridizing to a probe derived from the nucleotide sequence disclosed in Figure [2]1 under conditions of intermediate to high stringency, or (iii) being complementary to a nucleotide sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2).

18. A substantially purified BGL4 polypeptide with the biological activity of a β -glucosidase, comprising a sequence selected from the group consisting of:

- (a) an amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
- (b) an amino acid sequence having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
- (c) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
- (d) an amino acid sequence presented in Figure 2 (SEQ ID NO:2);
- (e) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;
- (f) an amino acid sequence presented as SEQ ID NO:2;
a substantially purified biologically active fragment of the amino acid sequence presented as SEQ ID NO:2.

25. A detergent composition, said composition comprising a polypeptide selected from the group consisting of:

- (a) an amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
- (b) an amino acid sequence having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
- (c) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
- (d) an amino acid sequence presented in Figure 2 (SEQ ID NO:2);
- (e) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;
- (f) an amino acid sequence presented as SEQ ID NO:2;

a substantially purified biologically active fragment of the amino acid sequence presented as SEQ ID NO:2.